MASHING PROCESS

FIELD OF THE INVENTION

The present invention relates, inter alia, to a mashing and filtration step in a process for the production of an alcoholic beverage, such as beer or whiskey, and to a composition useful in the mashing and filtration step in such a process.

BACKGROUND OF THE INVENTION

The use of enzymes in brewing is common. Application of enzymes to the mashing step to improve mash filterability and increase extract yield is described in WO 97/42302. However, there is a need for improvement of the mashing and filtration step and for improved enzymatic compositions for use in the mashing and filtration step.

SUMMARY OF THE INVENTION

The invention provides a process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise; a xylanase of glucoside hydrolase family 10 present in an amount of at least 15% w/w of the total xylanase and endoglucanase enzyme protein.

In a further aspect the invention provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one xylanolytic enzyme for its hydrolytic activity towards insoluble wheat arabinoxylan, selecting a xylanolytic enzyme which cleaves next to branched residues thereby leaving terminal substituted xylose oligosaccharides, and adding the selected xylanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

In an even further aspect the invention provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one endoglucanolytic enzyme for its hydrolytic activity towards barley beta-glucan, selecting a endoglucanolytic enzyme which under the conditions: 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan in 50 mM sodium acetate, 0.01% Triton X-100, at pH 5.5 and 50°C, within 1 hour degrades more than 70% of the barley beta-glucan to DP 6 or DP<6, and adding the selected endoglucanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

In yet a further aspect the invention provides a composition comprising; a GH10 xylanase present in an amount of at least 15% w/w of the total enzyme protein; and/or, a GH12, GH7 and/or GH5 endoglucanase present in an amount of at least 40% w/w of the total enzyme protein.

Other aspects include the use of the composition of the proceeding aspect in a process of comprising reduction of the viscosity of an aqueous solution comprising a starch

hydrolysate, including such processes wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making, or wherein the aqueous solution comprising a starch hydrolysate is intended for use in a feed composition.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Throughout this disclosure, various terms that are generally understood by those of ordinary skill in the arts are used. Several terms are used with specific meaning, however, and are meant as defined by the following.

As used herein the term "**grist**" is understood as the starch or sugar containing material that's the basis for beer production, e.g. the barley malt and the adjunct.

The term "**malt**" is understood as any malted cereal grain, in particular barley.

The term "**adjunct**" is understood as the part of the grist which is not barley malt. The adjunct may be any carbohydrate rich material.

The term "**mash**" is understood as a aqueous starch slurry, e.g. comprising crushed barley malt, crushed barley, and/or other adjunct or a combination hereof, steeped in water to make wort.

The term "**wort**" is understood as the unfermented liquor run-off following extracting the grist during mashing.

The term "**spent grains**" is understood as the drained solids remaining when the grist has been extracted and the wort separated from the mash.

The term "**beer**" is here understood as fermented wort, e.g. an alcoholic beverage brewed from barley malt, optionally adjunct and hops.

The term "**extract recovery**" in the wort is defined as the sum of soluble substances extracted from the grist (malt and adjuncts) expressed in percentage based on dry matter.

The term "**a thermostable enzyme**" is understood as an enzyme that under the temperature regime and the incubation period applied in the processes of the present invention in the amounts added is capable of sufficient degradation of the substrate in question.

The term "**Type A xylanase**" is understood as a xylanase that cleaves arabinoxylan polymers close to branched residues leaving terminal substituted xylose oligosaccharides. Type A xylanases may be identified using the method described in the Methods section of the present disclosure

The term "**homology**" when used about polypeptide or DNA sequences and referred to in this disclosure is understood as the degree of homology between two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP

provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453. The following settings for polypeptide sequence comparison are used: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

The term "**DP**" is the degree of polymerisation, herein used for average number of glucose units in polymers in a polysaccharide hydrolysate.

The numbering of **Glycoside Hydrolase Families** (GH) and **Carbohydrate Binding Modules** (CBM) applied in this disclosure follows the concept of Coutinho, P.M. & Henrissat, B. (1999) *CAZy - Carbohydrate-Active Enzymes server* at URL: http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html or alternatively Coutinho, P.M. & Henrissat, B. 1999; The modular structure of cellulases and other carbohydrate-active enzymes: an integrated database approach. In *"Genetics, Biochemistry and Ecology of Cellulose Degradation"*., K. Ohmiya, K. Hayashi, K. Sakka, Y. Kobayashi, S. Karita and T. Kimura eds., Uni Publishers Co., Tokyo, pp. 15-23, and in Bourne, Y. & Henrissat, B. 2001; Glycoside hydrolases and glycosyltransferases: families and functional modules, *Current Opinion in Structural Biology* 11:593-600. This classification system groups glucoside hydrolases based on similarities in primary structure. The members of a family furthermore show the same catalytic mechanism and have similarities in the overall three-dimensional structure, although a family may contain members with substantial variation in substrate specificity.

The naming of *Humicola insolens* endoglucanases follows the system of Karlsson, J. 2000. Fungal Cellulases, Study of hydrolytic properties of endoglucanases from *Trichoderma reesei* and *Humicola insolens*. Lund University.

Brewing processes are well-known in the art, and generally involve the steps of malting, mashing, and fermentation. In the traditional brewing process the malting serves the purpose of converting insoluble starch to soluble starch, reducing complex proteins, generating colour and flavour compounds, generating nutrients for yeast development, and the development of enzymes. The three main steps of the malting process are steeping, germination, and kilning.

Steeping includes mixing the barley kernels with water to raise the moisture level and activate the metabolic processes of the dormant kernel. In the next step, the wet barley is germinated by maintaining it at a suitable temperature and humidity level until adequate modification, i.e. such as degradation of starch and activation of enzymes, has been achieved. The final step is to dry the green malt in the kiln. The temperature regime in the kiln determines the colour of the barley malt and the amount of enzymes which survive for use in the mashing process. Low temperature kilning is more appropriate for malts when it is essential to preserve enzymatic activity. Malts kilned at high temperatures have very little or no enzyme activity but are very high in colouring such as caramelized sugars as well as in flavouring compounds.

Mashing is the process of converting starch from the milled barley malt and solid adjuncts into fermentable and unfermentable sugars to produce wort of the desired composition. Traditional mashing involves mixing milled barley malt and adjuncts with water at a set temperature and volume to continue the biochemical changes initiated during the malting process. The mashing process is conducted over a period of time at various temperatures in order to activate the endogenous malt enzymes responsible for the degradation of proteins and carbohydrates. By far the most important change brought about in mashing is the conversion of starch molecules into fermentable sugars. The principal enzymes responsible for starch conversion in a traditional mashing process are alpha- and beta-amylases. Alpha-amylase very rapidly reduces insoluble and soluble starch by splitting starch molecules into many shorter chains that can be attacked by beta-amylase. The disaccharide produced is maltose.

Traditionally lager beer has often been brewed using a method referred to as "step-infusion". This mashing procedure involves a series of rests at various temperatures, each favouring one of the necessary endogenous enzyme activities. To day the double-mash infusion system is the most widely used system for industrial production of beer, especially lager type beer. This system prepares two separate mashes. It utilizes a cereal cooker for boiling adjuncts and a mash tun for well-modified, highly enzymatically active malts.

When brewing from grists low in enzymes such as high adjunct grists, mashing may be performed in the presence of added enzyme compositions comprising the enzymes necessary for the hydrolysis of the grist starch. These enzymes may comprise alpha-amylases, pullulanases, beta-amylases and glucoamylases.

After mashing, it is necessary to separate the liquid extract (the wort) from the solids (spent grains i.e. the insoluble grain and husk material forming part of grist). Wort separation is important because the solids contain large amounts of non-starch polysaccharides, protein, poorly modified starch, fatty material, silicates, and polyphenols (tannins). Important non-starch polysaccharides present in cereal grains are beta-glucan and arabinoxylan. The endosperm cell wall of barley comprises 75% beta-glucan, 20% arabinoxylan, and 5% remaining protein with small amount of cellulose, glucomannan and phenolic acids. Long chains of barley arabinoxylans, and to a lesser degree beta-glucan, which have not been modified due to enzymatic hydrolysis may cause formation of gels when solubilised in water, these gels will strongly increase wort viscosity and reduce filterability. Likewise is it very important for the quality of the wort that the beta-glucan has been reduced to smaller oligomers, as unmodified beta-glucans later on will give rise to haze stability problems in the final beer. Therefore, enzymatic compositions comprising endoglucanases and xylanases, such as Ultraflo® or Viscozyme®, are often used in the mashing step to improve wort separation. The objectives of wort separation, *inter alia*, include the following:

- to obtain good extract recovery,

- to obtain good filterability, and
- to produce clear wort.

Extraction recovery and filterability are important for the economy in the brewing process, while the wort clarity is a must in order to produce a beer which does not develop haze. Extraction recovery, filterability and wort clarity is greatly affected by the standard of the grist, e.g. the barley malt and the types of adjunct, as well as the applied mashing procedure.

Following the separation of the wort from the spent grains the wort may be fermented with brewers yeast to produce a beer.

Further information on conventional brewing processes may be found in "Technology Brewing and Malting" by Wolfgang Kunze of the Research and Teaching Institute of Brewing, Berlin (VLB), 2nd revised Edition 1999, ISBN 3-921690-39-0.

Embodiments of the invention

The invention provides a process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise; a xylanase of GH family 10 present in an amount of at least 15%, 20%, preferably 25%, such as at least 30%, or at least 40%, at least 50% or at least 60% such as at least 70%, at least 80%, at least 90%, or even 100% w/w of the total xylanase and endoglucanase enzyme protein.

In a preferred embodiment the xylanase is a type A xylanase, and in a particular embodiment the xylanase is a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60.

Preferably the xylanase has a CBM, preferably a CBM of family 1.

In another preferred embodiment the xylanase is a xylanase which in the xylanase binding assay described herein has a barley soluble/insoluble fibre binding ratio of at least 0.50, preferably at least 0.60, more preferably at least 0.70, such as 0.80, 0.90, 1.00, 1.10 or even at least 1.20.

In another preferred embodiment the xylanase is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:8 or SEQ ID NO:9), from a strain of a *Myceliophotora* sp., preferably from a *Myceliophotora thermophilia* (SEQ ID NO:13), from a strain of a *Humicola* sp., preferably from *Humicola insolens* (SEQ ID NO:12). In yet another preferred embodiment the xylanase is derived from a strain of a Trichoderma sp., preferably from T. reesei such as the xylanase shown in SEQ ID NO:17 In a more preferred embodiment the xylanase the xylanase is derived from a has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In another preferred embodiment the xylanase is derived from a bacterium such as from a strain of a *Bacillus*, preferably from *Bacillus halodurans*.

In another preferred embodiment the endoglucanase is an endoglucanase derived from *Humicola* sp., such as the endoglucanase from *Humicola insolens* (SEQ ID NO:3), or the endoglucanase from *H. insolens* (SEQ ID NO:4), from *Thermoascus* sp., such as the endoglucanase derived from *Thermoascus aurantiacus* (SEQ ID NO:6), or from *Aspergillus* sp., such as the endoglucanase derived from *Aspergillus aculeatus* (SEQ ID NO:16).

In a preferred embodiment the xylanase has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In another preferred embodiment at least one additional enzyme is present, which enzyme is arabinofuranosidase.

The invention also provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one xylanolytic enzyme for its hydrolytic activity towards insoluble wheat arabinoxylan, selecting a xylanolytic enzyme which cleaves next to branched residues thereby leaving terminal substituted xylose oligosaccharides, and adding the selected xylanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

The invention further provides a process of reducing the viscosity of an aqueous solution comprising a starch hydrolysate, said process comprising: testing at least one endoglucanolytic enzyme for its hydrolytic activity towards barley beta-glucan, selecting a endoglucanolytic enzyme which under the conditions: 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan in 50 mM sodium acetate, 0.01% Triton X-100, at pH 5.5 and 50°C, within 1 hour degrades more than 70% of the barley beta-glucan to DP 6 or DP<6, and adding the selected endoglucanolytic enzyme to the aqueous solution comprising a starch hydrolysate.

In preferred embodiments of the two processes the aqueous solution comprising a starch hydrolysate is a mash for beer making.

The invention also provides a composition comprising; a GH10 xylanase present in an amount of at least 15% w/w of the total enzyme protein; and/or, a GH12, GH7 and/or GH5 endoglucanase present in an amount of at least 40% w/w of the total enzyme protein.

In a preferred embodiment the xylanase of the composition is a type A xylanase, and preferably a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60.

In a preferred embodiment the xylanase of the composition is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:8 or SEQ ID NO:9), from a strain of a *Myceliophotora* sp., preferably from a *Myceliophotora thermophilia* (SEQ ID NO:13), from a strain of a *Humicola* sp., preferably from *Humicola insolens* (SEQ ID NO:12). In a preferred embodiment the xylanase of the composition has at least 50%, such as at least 60%, 70%, 80% or even 90% homology

to any of the aforementioned sequences.

In a preferred embodiment the xylanase of the composition is derived from a bacterium such as from a strain of a *Bacillus*, preferably from *Bacillus halodurans*.

In a preferred embodiment the endoglucanase of the composition is an endoglucanase derived from *Humicola* sp., such as the endoglucanase from *Humicola insolens* (SEQ ID NO:3), the endoglucanase from *H. insolens* (SEQ ID NO:4) or from *Thermoascus* sp., such as the endoglucanase derived from *Thermoascus aurantiacus* (SEQ ID NO:6), or from *Aspergillus* sp., such as the endoglucanase derived from *Aspergillus aculeatus* (SEQ ID NO:16), or from Trichoderma sp. preferably from T. reesei and/or T. viride, such as the family 5 endoglucanase shown in SEQ ID NO:18, the family 7, beta-glucanase shown in SEQ ID NO:19 or the fam 12, beta-glucanase shown in SEQ ID NO:20

In a preferred embodiment the endoglucanase of the composition has at least 50%, such as at least 60%, 70%, 80% or even 90% homology to any of the aforementioned sequences.

In a preferred embodiment the xylanase GH family 10 of the composition is present in an amount of at least 20%, preferably at least 25%, such as at least 30%, at least 35%, at least 40%, at least 45% or even at least 50% w/w of the total xylanase and endoglucanase enzyme protein.

In a preferred embodiment the endoglucanase of GH Family 12, 7 and/or 5 endoglucanase of the composition is present in an amount of at least 25%, preferably 30%, such as at least 35%, at least 40%, at least 45% or even at least 50%, such as at least 55%, or even at least 60% w/w of the total xylanase and endoglucanase enzyme protein.

The composition according to the proceeding aspect may be used in a process comprising reducing the viscosity of an aqueous solution comprising a starch hydrolysate.

The composition may even be used in a process comprising filtering of an aqueous solution comprising a starch hydrolysate. In a preferred embodiment the aqueous solution comprising a starch hydrolysate is a mash for beer making, and in another preferred embodiment the aqueous solution comprising a starch hydrolysate is a feed composition.

The process of the invention may be applied in the mashing of any grist. According to the invention the grist may comprise any starch and/or sugar containing plant material derivable from any plant and plant part, including tubers, roots, stems, leaves and seeds. Preferably the grist comprises grain, such as grain from barley, wheat, rye, oat, corn, rice, milo, millet and sorghum, and more preferably, at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of the wort is derived from grain. Most preferably the grist comprises malted grain, such as barley malt. Preferably, at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of the wort is derived from malted grain.

For mashing of low malt grists the mashing enzymes may be exogenously supplied. The enzymes mostly used as starch degrading enzymes include pullulanases, alpha-amylases and amyloglucosidases. The use of starch degrading enzymes in mashing is well-known to the skilled person.

Adjunct comprising readily fermentable carbohydrates such as sugars or syrups may be added to the malt mash before, during or after the mashing process of the invention but is preferably added after the mashing process. A part of the adjunct may be treated with a protease and/or a endoglucanase, and/or heat treated before being added to the mash of the invention.

During the mashing process, starch extracted from the grist is gradually hydrolyzed into fermentable sugars and smaller dextrins. Preferably the mash is starch negative to iodine testing, before wort separation.

The application of the appropriate xylanase and endoglucanase activities in the process of the present invention results in efficient reduction of beta-glucan and arabino-xylan level facilitating wort separation, thus ensuring reduced cycle time, high extract recovery and clear wort.

The wort produced by the process of the first aspect of the invention may be fermented to produce a beer. Fermentation of the wort may include pitching the wort with a yeast slurry comprising fresh yeast, i.e. yeast not previously used for the invention or the yeast may be recycled yeast. The yeast applied may be any yeast suitable for beer brewing, especially yeasts selected from *Saccharomyces* spp. such as *S. cerevisiae* and S. *uvarum*, including natural or artificially produced variants of these organisms. The methods for fermentation of wort for production of beer are well known to the person skilled in the arts.

The process of the invention may include adding silica hydrogel to the fermented wort to increase the colloidal stability of the beer. The processes may further include adding kieselguhr to the fermented wort and filtering to render the beer bright. The beer produced by fermenting the wort of the invention may be any type of beer, e.g. ale, strong ale, stout, porter, lager, pilsner, bitter, export beer, malt liquor, happoushu, lambic, barley wine, high-alcohol beer, low-alcohol beer, low-calorie beer or light beer.

The beer produced by the process of the invention may be distilled to recover ethanol, e.g. for whisky production. Contemplated are any kind of whisky (spelled "whiskey" in US and Ireland) include bourbon, Canadian whisky, Irish whiskey, rye, and scotch.

Xylanase

For the present purposes a xylanase is an enzyme classified as EC 3.2.1.8. The official name is endo-1,4-beta-xylanase. The systematic name is 1,4-beta-D-xylan xylanohydrolase. Other names may be used, such as endo-(1-4)-beta-xylanase; (1-4)-beta-xylan 4-xylanohydrolase; endo-1,4-xylanase; xylanase; beta-1,4-xylanase; endo-1,4-

xylanase; endo-beta-1,4-xylanase; endo-1,4-beta-D-xylanase; 1,4-beta-xylan xylanohydrolase; beta-xylanase; beta-1,4-xylan xylanohydrolase; endo-1,4-beta-xylanase; beta-D-xylanase. The reaction catalysed is the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans.

While the xylanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the xylanase is of microbial origin. In particular the xylanase may be one derivable from a filamentous fungus or a yeast.

Xylanases have been found in a number of fungal species, in particular species of *Aspergillus*, such as *A. niger*, *A. awamori*, *A. aculeatus and A. oryzae*, *Trichoderma*, such as *T. reesei* or *T. harzianum, Penicillium*, such as *P. camenbertii, Fusarium*, such as *F. oxysporum*, *Humicola*, such as *H. insolens,* and *Thermomyces lanuginosa,* such as *T. lanuginosa.* Xylanases have also been found in bacterial species, e.g. within the genus *Bacillus*, such as *B. pumilus.*

Preferably, according to the process of the invention the xylanase is derived from a filamentous fungus such as from *Aspergillus* sp., *Bacillus sp., Humicola* sp., *Myceliophotora* sp., *Poitrasia* sp. *Rhizomucor* sp. or *Trichoderma*.

Substrate specificity was shown to be a key parameter for the performance of xylanases in the process of the invention. A xylanase with optimum performance in the process of the invention seems to be an enzyme which binds rather strongly to soluble arabino-xylan and rather weakly to insoluble arabino-xylan. Preferably the xylanase to be used in the present invention is a xylanase which in the binding assay in the Methods description of this disclosure has a barley soluble/insoluble fibre binding ratio of at least 0.50, preferably at least 0.60, more preferably at least 0.70, such as 0.80, 0.90, 1.00, 1.10 or even at least 1.20.

A number of xylanases identified having these characteristics are members of the glucoside hydrolase family 10. Preferably the xylanase to be used in the present invention is a Glycoside Hydrolase Family 10 (GH10) xylanase, and most preferably the xylanase is a GH10 xylanase which is also a type A xylanase i.e. a xylanase which cleaves insoluble wheat arabinoxylan polymers close to branched residues leaving terminal substituted xylose oligosaccharides (please see the examples for a definition of type A and B). As the GH10 enzymes are able to go closer to the branched xylose units, they form smaller oligosaccharides than the GH11 xylanases.

Preferably the xylanase to be used in the present invention has a functional CBM, such as a CBM of family 1.

Preferably, according to the process of the invention the xylanase is selected from the list consisting of the xylanase from shown as, the xylanase from *Aspergillus aculeatus* shown as SEQ ID NO:8 (AA XYL I), the xylanase from *Aspergillus aculeatus* shown as SEQ ID NO:9 (AA XYL II), the xylanase from *Bacillus halodurans* shown as SWISS PROT P07528 (BH XYL A), the xylanase from *Humicola insolens* shown as SEQ ID NO:12 (HI XYL III), the

xylanase from *Myceliophotora thermophila* shown as SEQ ID NO:13 (MT XYL I), and the xylanase from *Trichoderma reesei*, such as the xylanase shown as SEQ ID NO:17. Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned xylanase sequences.

Endoglucanase

For the present purposes an endoglucanase is an enzyme classified as EC 3.2.1.4. While the endoglucanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the endoglucanase is of microbial origin. In particular the endoglucanase may be one derivable from a filamentous fungus or a yeast.

Preferably the endoglucanase is a Glycoside Hydrolase Family 12 (GH12), Glycoside Hydrolase Family 7 (GH7) or a Glycoside Hydrolase Family 5 (GH5) glucanase. More preferably the endoglucanase is a polypeptide having a beta-jelly-roll or a b8/a8-barrell in superstructure.

While the endoglucanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the endoglucanase is of microbial origin. In particular the endoglucanase may be one derivable from a filamentous fungus or a yeast.

More preferably, according to the process of the invention the endoglucanase is derived from a filamentous fungus such as from *Aspergillus* sp. or *Humicola* sp.

Preferably, according to the process of the invention the endoglucanase is selected from the list consisting of the endoglucanase from *Aspergillus aculeatus* shown in SEQ ID NO:1 (AA EG I), the endoglucanase from *Aspergillus aculeatus* shown in SEQ ID NO:2 (AA EG II), the endoglucanase from *Aspergillus aculeatus* shown in SEQ ID NO:16 (AA EG III), the endoglucanase from *Humicola insolens* shown in SEQ ID NO:3 (HI EG I), the endoglucanase from *Humicola insolens* shown in SEQ ID NO:4 (HI EG III), the endoglucanase from *Humicola insolens* shown in SEQ ID NO:5 (HI EG IV), the endoglucanase from Trichoderma sp. shown in SEQ ID NO:18, the endoglucanase from Trichoderma sp. shown in SEQ ID NO:19 or the endoglucanase from Trichoderma sp. shown in SEQ ID NO:20. Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned sequences.

Other GH12 glucanases includes endoglucanases obtained from *Aspergillus* sp. such as from *Aspergillus kawachii* (SWISSPROT Q12679), or *Aspergillus niger* (SWISSPROT O74705), *Aspergillus oryzae* (SWISSPROT O13454), from *Erwinia* sp., such as from *Erwinia carotovora* (SWISSPROT P16630), and from *Thermotoga* sp., such as from *Thermotoga maritima* (SWISSPROT Q60032 or Q9S5X8). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of

the aforementioned GH12 glucanases sequences.

Other GH7 glucanases includes endoglucanases obtained from *Agaricus* sp., such as from *Agaricus bisporus* (SWISSPROT Q92400), from *Aspergillus* sp., such as from *Aspergillus niger* (SWISSPROT Q9UVS8), from *Fusarium* sp., such as from *Fusarium oxysporum* (SWISSPROT P46238), from *Neurospora* sp., such as from *Neurospora crassa* (SWISSPROT P38676), and from *Trichoderma* sp., such as from *Trichoderma longibrachiatum* (SWISSPROT Q12714). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned GH7 glucanases sequences.

Other GH5 glucanases includes endoglucanases obtained from *Acidothermus* sp., such as from *Acidothermus cellulolyticus* (SWISSPROT P54583), from *Aspergillus* sp., such as from *Aspergillus niger* (SWISSPROT O74706), and from *Bacillus* sp., such as from *Bacillus polymyxa* (SWISSPROT P23548). Also preferred are any sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned GH5 glucanases sequences.

Arabinofuranosidase

Arabinofuranosidase EC 3.2.1.55, common name alpha-N-arabinofuranosidase hydrolysise terminal non-reducing alpha-L-arabinofuranoside residues in alpha-L-arabinosides. The enzyme acts on alpha-L-arabinofuranosides, alpha-L-arabinans containing (1,3)- and/or (1,5)-linkages, arabinoxylans and arabinogalactans.

MATERIALS AND METHODS

Xylanase activity

The xylanolytic activity can be expressed in FXU-units, determined at pH 6.0 with remazol-xylan (4-O-methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R, Fluka) as substrate.

A xylanase sample is incubated with the remazol-xylan substrate. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant (as determined spectrophotometrically at 585 nm) is proportional to the xylanase activity, and the xylanase units are then determined relatively to an enzyme standard at standard reaction conditions, i.e. at 50.0 °C, pH 6.0, and 30 minutes reaction time.

A folder AF 293.6/1 describing this analytical method in more detail is available upon request to Novozymes A/S, Denmark, which folder is hereby included by reference.

Glucanase activity

The cellulytic activity may be measured in fungal endoglucanase units (FBG), determined on a 0.5% beta-glucan substrate at 30°C, pH 5.0 and reaction time 30 min. Fungal

endoglucanase reacts with beta-glucan releases glucose or reducing carbohydrate which is determined as reducing sugar according to the Somogyi-Nelson method.

1 fungal endoglucanase unit (FBG) is the amount of enzyme which according to the above outlined standard conditions, releases glucose or reducing carbohydrate with a reduction capacity equivalent to 1 micromol glucose per minute.

Enzymes

Ultraflo® L, a multicomponent enzyme composition derived from *Humicola insolens* comprising a mixture of endoglucanases, xylanases, pentosanases and arabanases. Ultraflo® L is standardized to 45 FBG/g, and has a gravity of approximately 1.2 g/ml. Ultraflo® is available from Novozymes A/S.

Viscozyme® L, a multicomponent enzyme composition derived from *Aspergillus aculeatus* comprising a mixture of endoglucanases, arabanases and xylanases. Viscozyme® L is standardized to 100 FBG/g, and has a gravity of approximately 1.2 g/m l. Viscozyme is available from Novozymes A/S.

Alcalase®, Subtilisin a protease composition derived from *Bacillus licheniformis*. Alcalase® is available from Novozymes A/S.

Termamyl SC ®, a *Bacillus* alpha-amylase available from Novozymes A/S.

The following monocomponent endoglucanases and xylanases were applied:

Endoglucanases;			
AA EG I	*Aspergillus aculeatus*		SEQ ID NO:1
AA EG II	*Aspergillus aculeatus*	Cel12b	SEQ ID NO:2
AA EG III	*Aspergillus aculeatus*	Cel12a	SEQ ID NO:16
HI EG I	*Humicola insolens*	Cel12a, GH12	SEQ ID NO:3.
HI EG III	*Humicola insolens*	Cel12a, GH12	SEQ ID NO:4.
HI EG IV	*Humicola insolens*	Cel5a, GH12	SEQ ID NO:5
HI EG V	*Humicola insolens*	Cel45a, GH45	SEQ ID NO:8
TA EG BG025	*Thermoascus aurantiacus*		SEQ ID NO:6
Xylanases			
AA XYL I	*Aspergillus aculeatus*	GH10, Type A	SEQ ID NO:8
AA XYL II	*Aspergillus aculeatus*	GH10, Type A	SEQ ID NO:9
AA XYL III	*Aspergillus aculeatus*	GH11, Type B	SEQ ID NO:10
BH XYL A	*Bacillus halodurans*	GH10, Type A	SWISS PROT P07528.
HI XYL I	*Humicola insolens*	GH11, Type B	SEQ ID NO:11
HI XYL III	Humicola insolens	GH10, Type A	SEQ ID NO:12
MT XYL I	*Myceliophotora thermophila*	GH10, Type A	SEQ ID NO:13
MT XYL III	*Myceliophotora thermophila*	GH11, Type B	SEQ ID NO:14
TL XYL	*Thermomyces lanuginosus*	GH11, Type B	SEQ ID NO:15

Methods

Mash preparation

Unless otherwise stated mashing was performed as follows. Except when noted (e.g. with regard to enzyme dosage) the mash was prepared according to EBC: 4.5.1 using malt grounded according to EBC: 1.1. Mashing trials were performed in 500 ml lidded vessels incubated in water bath with stirring and each containing a mash with 50 g grist and adjusted to a total weight of 300±0.2 g with water preheated to the initial incubation temperature + 1°C. The wort produced was app. 12% Plato.

Mashing temperature profile

Unless otherwise stated mashing was carried out using an initial incubation temperature at 52°C for 30 minutes, followed by an increasing step to 63°C remaining here for 20 min. The profile is continued with an increasing step to 72°C for 30 min, and mashing off at 78°C for 5 min. All step wise temperature gradients are achieved by an increase of 1°C/min. The mash is cooled to 20°C during 15 min, which result in a total incubation period

of 2 hours and 11 min.

Additional methods

Methods for analysis of raw products, wort, beer etc. can be found in *Analytica-EBC*, Analysis Committee of EBC, the European Brewing Convention (1998), Verlag Hans Carl Geranke-Fachverlag. For the present invention the methods applied for determination of the following parameters were as indicated below.

Plato: refractometer.

Beta-glucan: EBC: 8.13.2 (High Molecular weight beta-glucan content of wort: Fluorimetric Method).

Turbidity: EBC: 4.7.1

Filterability: Volume of filtrate (ml) determination: According to EBC: 4.5.1 (Extract of Malt: Congress Mash) subsection 8.2. Filterability: Filtration volume is read after 1 hour of filtration through fluted filter paper, 320 mm diameter. Schleicher and Schüll No.597 ½, Machery, Nagel and Co. in funnels, 200 mm diameter, fitted in 500 ml flasks.

Extract recovery: EBC: 4.5.1 (Extract of Malt: Congress Mash, Extract in dry). The term extract recovery in the wort is defined as the sum of soluble substances (glucose, sucrose, maltose, maltotriose, dextrins, protein, gums, inorganic, other substances) extracted from the grist (malt and adjuncts) expressed in percentage based on dry matter. The remaining insoluble part is defined as spent grains.

$$a)\ E_1 = \frac{P(M+800)}{100-P}$$

$$b)\ E_2 = \frac{E_1 \cdot 100}{100-M}$$

where;

E_1 = the extract content of sample, in % (m/m)

E_2 = the extract content of dry grist, in % (m/m)

P = the extract content in wort, in % Plato

M = the moisture content of the grist, in % (m/m)

800 = the amount of destilled water added into the mash to 100 g of grist

Viscosity: Automated Microviscometer (AMVn) is based on the rolling ball principle. The sample to be measured is introduced into a glass capillary in which a steel ball rolls. The viscous properties of the test fluid can be determined by measuring the rolling time of the steel ball. The rolling time t_0 of a ball over a defined measuring distance in a capillary is measured. The dynamic viscosity η of the sample is calculated from the calibration constant $K_1(\alpha)$ of the measuring system, the rolling time t_0 and the difference of density $\Delta\rho$ between the ball and the sample. The following equation is used:

$$\eta = K_1(\alpha) \cdot t_0 \cdot (\rho_k - \rho_s), \text{ where}$$

η = *Dynamic viscosity of the sample,* $[mPa \cdot s]$
$K(\alpha)$ = *Calibration* constant *for the Measuring system* $[mPa \cdot scm^3 / g]$
t_0 = *Rolling time for* $100mm$ $[s]$
ρ_k = *Ball density* $[7{,}85 g / cm^3]$
ρ_s = *Densityof the sample measured* $[g / cm^3]$

The viscosity is presented based on the extract (Plato°) as is, or converted to 8,6°Plato based upon a Congress mashing procedure.

Example 1. Characterisation of xylanases using binding assay

Production of fibre fractions

Soluble fibre fraction of barley was produced as follows:

1. 50 kg of barley was milled and slurred into 450 kg water at 50°C under stirring.
2. The extraction was carried out for 30 minutes under stirring.
3. Using a preheated decanter centrifuge at 50°C, and a solids ejecting centrifuge a particle free and clarified fraction was prepared.
4. The clarified fraction was ultra filtered at 50°C on a tubular membrane with a cut-off value of 20000 Dalton. The ultra filtration process was continued until the viscosity increased and the flow was reduced significantly in the system.
5. The concentrated fraction was collected and lyophilized.

Insoluble fibre fraction of barley was produced as follows:

1. 50 kg of barley was milled and slurred into 450 kg of water at 50°C. 0.25 kg of Termamyl SC was added and the solution was heated to 85°C under stirring. The reaction was carried out for 30 minutes. A sample was taken for starch analysis by iodine test.
2. The sample was centrifuged for 5 min at 3000 x g (in 10 ml centrifuge vial). °Plato was measured by using a refractometer on the supernatant. Starch conversion was followed by iodine colour reaction; if blue starch was remaining.
3. The reaction was continued until °Plato has stabilized. The reaction product was ready for centrifugation.
4. The centrifugation was carried out using a decanter. The separation was carried out at 75°C, and a clear and particle free supernatant was obtained. This fraction was discarded. Only the solid fraction was used in the following process.
5. The collected solid fraction was slurred into 500 kg of hot water. The temperature of this slurry was adjusted to 50°C.
6. pH was adjusted to 7.5 using NaOH. A hydrolysis reaction was carried out using 125

g Alcalase 2.4 L. During the hydrolysis pH was maintained at pH=7.5 (pH-stat) and the reaction time was 120 minutes. Hereafter the reaction was left stirred without pH-stat at T=50°C over night.

7. pH was then adjusted to 6.5 using HCl.
8. The reaction mixture was centrifuged using the decanter.
9. The solid fraction was collected and washed with 500 L of water at 50°C for 30 minutes. The centrifugation step and washing step was repeated.
10. This washed solid fraction was lyophilized.

Fibre fraction analysis

The sugar composition of the fibre fractions was analysed as follows: 1 g of fibre was added 50 mL of 1 M HCl and incubated at 100 °C for 2 hours with shaking. After this treatment the reaction mixture was immediately cooled on ice and 11 mL of 4 M NaOH was added to neutralise the mixture. The content of arabinose, galactose, glucose and xylose was quantified using a Dionex BioLC system equipped with a CarBoPac PA-1 column as described in Sørensen et al. (2003) Biotech. Bioeng. vol. 81, No. 6, p. 726-731. The results are shown in table 1.

Table 1. Content (g/kg) of the individual sugars in the fibre fractions from barley

	Arabinose	Galactose	Glucose	Xylose
Soluble fibers	34.9	14.8	486.6	38.1
Insoluble fibers	102.3	10.4	42.3	207.2

Xylanases binding assay

The xylanases binding assay was performed as follows: The fibre (10 mg) was washed in an Eppendorf tube by whirly-mixing with 500 microL of acetate buffer (50 mM, pH 5.5, 0.1 % Triton X-100) before being centrifuged for 2 min at 13000 g. Washing and centrifuging was performed twice. The solution containing the enzyme* (500 microL, in acetate buffer pH 5.5) was then added to the substrate and the mixture was thoroughly whirly-mixed and kept in an ice bath for 10 min. The Eppendorf tube containing the reaction mixture was then centrifuged at14000 g for 3 min where after initial and residual activity was determined by using as substrate 0.2% AZCL-Arabinoxylan from wheat (Megazyme) in 0.2 M Na-phosphate buffer pH 6.0 + 0.01% Triton-x-100. A vial with 900 microL substrate was preheated to 37°C in a thermomixer. 100 microL enzyme sample was added followed by incubation for 15 min at 37°C and maximum shaking. The vial was placed on ice for 2 min before being centrifuged for 1 min at 20.000 g. From the supernatant 2 x 200 microL was transferred to a microtiter plate and endpoint OD 590 nm was measured and compared

relative to a control. The control was 100 microL enzyme sample incubated with 900 microL 0.2 M Na-phosphate buffer pH 6.0 + 0.01% Triton-x-100 instead of substrate and subsequently all activity is recovered in the supernatant and this value set to 1. The results are shown in table 2.

The two xylanases having the highest soluble/insoluble barley fibre binding ratio, Xylanase II and I from *A. aculeatus*, were also the two xylanases having the best performance in the mashing trials.

Table 2. Soluble/insoluble barley fibre binding ratio. Relative activity measured in the supernatant after 10 min incubation with soluble and insoluble barley fibre fractions and the resulting ratios between activities measured in the supernatants over soluble and insoluble barley fibre.

Xylanase	GH Family	Insoluble barley fibers	Soluble barley fibers	Ratio Soluble/
Aspergillus aculeatus Xyl II	10	86	104	1.21
Aspergillus aculeatus Xyl I	10	105	56	0.52
Humicola insolens Xyl II	11	82	37	0.45
Thermomyces lanuginosus Xyl	11	83	14	0.17
Humicola insolens Xyl I	11	74	7	0.09
Bacillus halodurans Xyl A	11	79	7	0.09

Example 2. Characterization of xylanase specificity

High field Nuclear Magnetic Resonance (^{1}H NMR) was applied to identify differences in xylanase specificity towards insoluble wheat arabinoxylan (AX) (insoluble, Megazyme).

In ^{1}H NMR, arabinoxylan or oligosaccharides hereof (AXO) show signals (chemical shifts) around 5.0-5.5 ppm arising from the anomeric protons H-1 from the α-L-arabinofuranoside units. The individual differences among these depending on their local surroundings can be used to evaluate the specificity of xylanases towards this highly branched polymer.

The standard condition was 10 mg/mL of AX in 50 mM acetate buffer, pH 5.5 was incubated with 0.1 XU/mL for 120 min at 30°C. The xylanase was then inactivated (95°C, 20 min) and the solution concentrated on a rotary evaporator. The sample was then evaporated twice from D_2O (1 mL) and finally re-suspended in D_2O (~0.8 mL) before being analyzed. ^{1}H NMR spectra were recorded on a Varian Mercury 400 MHz at 30°C. Data were collected over 100 scans and the HDO signal was used as a reference signal (4.67 ppm).

Degradation of AX with a xylanase changes the ^{1}H NMR spectra according to the specificity of the enzyme. Thus, the chemical shift of the arabinofuranoside H-1 changes if the arabinose in the resulting oligosaccharide is located on a terminal xylose as compared to an "internal" xylose. This will be the result if the xylanase is capable at placing a substituted

xylose unit in its +1 subsite. Using the applied conditions it was found that all tested GH10 xylanases was able to do this, whereas no GH11 xylanases having the characteristic were found. Type A refers to a xylanase that cleaves next to branched residues (leaving terminal substituted xylose oligosaccharides) whereas Type B refers to a xylanase that cleaves between unsubstituted xylose units giving internal substituted units only. Type A xylanases are also capable at cleaving between unsubstituted xylose units. Examples of Type A and Type B xylanase identified by the inventors are shown in table 3. For the invention a type A xylanase is preferred.

Table 3. Examples of Type A and Type B xylanases

Type A	Type B
Aspergillus aculeatus Xyl I	Biobake (Quest)
Aspergillus aculeatus Xyl II	*Humicola insolens* Xyl I
Bacillus halodurans Xyl A	*Myceliophotora thermophila* Xyl III
Humicola insolens Xyl III	*Thermomyces lanuginosus* Xyl I
Myceliophotora thermophila Xyl I	

Even within the type A xylanases the preference for cleavage next to branched residues or between unsubstituted xylose varies as shown in table 4, where the ratio $I_{1,3terminal}/I_{1,3internal}$ relates to the ratio between the respective integrals of the two types of protons. Thus, type A cleavage result in an increase of $I_{1,3terminal}$ whereas type B does not. The chemical shifts for the two types of protons are: 1,3-linked arabinofuranoside H-1 on terminal xylose : 5.26 ppm and 1,3-linked arabinofuranoside H-1 on internal xylose: 5.32 ppm. For the invention a type A xylanase having a $I_{1,3terminal}/I_{1,3internal}$ ratio of at least 0.25, such as at least 0.30, al least 0.40, at least 0.50, or even at least 0.60, is preferred.

Table 4. Xylanases specificity, preference for cleavage next to branched residues or between unsubstituted xylose

	$I_{1,3terminal}/I_{1,3internal}$
Myceliophotora thermophila Xyl I	0.64
Aspergillus aculeatus Xyl II	0.60
Humicola insolens Xyl III	0.30
Aspergillus aculeatus Xyl I	0.28

Example 3. Characterization of endoglucanase specificity

Specificity of endoglucanases was studied by analyzing degradation products upon incubation with barley beta-glucan. Eppendorf tubes with 0.1 and 10 microgram/ml purified enzyme and 5 mg/ml barley beta-glucan (Megazyme, low viscosity) in 50 mM sodium acetate, 0.01% Triton X-100 at pH 5.5 were incubated in an Eppendorf thermomixer at 50°C with agitation.

Enzymes tested were *endoglucanase* EG I from *Humicola insolens*, endoglucanase EG III from *Humicola insolens*, endoglucanase *Humicola insolens* EG IV, *Aspergillus aculeatus* EG II (XG5, Cel12B), and *Aspergillus aculeatus* EG III (XG53, Cel12A).

Samples were withdrawn between 1 and 21.5 hours and inactivated by heating for 30 min at 95°C. Half the volume of each sample was degraded with lichenase (0.085 microgram/ml, Megazyme, from Bacillus subtilis) in 50 mM MES, 1 mM CaCl2, pH 6.5 for 2 hours at 50°C, after which the lichenase was inactivated by heating to 95°C for 30 min. Samples with and without lichenase treatment were diluted appropriately with Milli Q water and analyzed on a Dionex DX-500 HPAEC-PAD system (CarboPac PA-100 column; A buffer: 150 mM NaOH; B buffer: 150 mM NaOH + 0.6 M sodium acetate; Flow rate: 1 ml/min. Elution conditions: 0-3 min: 95% A + 5% B; 3-19 min: linear gradient: 95% A+ 5% B to 50% A and 50% B; 19-21 min: linear gradient: 50% A + 50% B to 100% B; 21-23 min: 100% B). As reference on the Dionex system a mixture of cellooligosaccharides was used (DP1 to DP6, 100 microM of each). Peaks in chromatograms were identified using the cellooligo references and known composition of barley beta-glucan after lichenase treatment (e.g. Izydorczyk, M.S., Macri, L.J., & MacGregor, A.W., 1997, Carbohydrate Polymers, 35, 249-258). Quantification of peaks in chromatograms was done using response factors obtained for cellooligo references and assuming that response factor was identical for oligosaccharides of same DP with beta-1,3 bonds. For oligosaccharides larger than DP6 response factor of DP6 was used.

From the analysis of degradation products with EG I from *Humicola insolens* (Tables 5 and 6), it was found that the enzyme is able to degrade both beta-1,3 and beta-1,4 bonds. Initially, cellobiose, cellotriose and to some extent laminaribiose are the main products increasing after lichenase treatment. This indicates that beta-1,3 bonds are accepted between glucose units in subsites -4/-3, -5/-4 and +1/+2. The main products with highest enzyme dosage (10 microgram/ml) and longest incubation time (21.5 hours) were found to be glucose and cellobiose.

With EG III from *Humicola insolens* (Tables 7 and 8) the main products after 21.5 hours and 10 microgram/ml enzyme were tetraoses (mainly Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu and Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu but not Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu), pentaoses (probably mainly Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu and Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu) and larger oligomers. Composition of degradation products after lichenase treatment shows that the enzyme exclusively degrades the beta-1,4 bonds in beta-glucan. Futhermore, the the beta-1,4 linkages that are hydrolysed are mainly those not hydrolysed by lichenases (without adjacent beta-1,3 bond towards the non-reducing end). That the amount of Glu(beta-1,4)Glu(beta-1,3)Glu ("Lic3") after lichenase treatment does not decrease significantly even after 21.5 hours with 10 microgram/ml indicates that the enzyme only has limited activity on stretches with only two beta-1,4 bonds between beta-1,3 linkages. The

appearance of significant amounts of glucose and laminaribiose but not cellobiose or cellotriose after lichenase treatment indicates that beta-1,3 bonds are accepted between glucose units in subsites -3/-2 and +1/+2 but not between -4/-3 or -5/-4.

The enzyme EG IV from *Humicola insolens* mainly degrades the beta-glucan to larger oligomers (Tables 9 and 10), but after 21.5 hours with 10 microgram/ml enzyme substantial amounts of cellobiose and oligomers of DP4 (probably mainly Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu and Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu) are formed. The enzyme degrades about equal amounts of beta-1,4 and beta-1,3 bonds in beta-glucan and the beta-1,4 bonds cleaved seem to be those without an adjacent beta-1,3 bond towards the non-reducing end (unlike lichenases). Lichenase treatment gives increased cellotriose already after limited hydrolysis with EG IV, whereas cellobiose and glucose only appear after more extensive hydrolysis with EG IV. This indicates that beta-1,3 bonds are better accepted between glucose in subsites -5/-4 than between -4/-3 and especially -3/-2. The appearance of laminaribiose after lichenase treatment shows that beta-1,3 bonds are also accepted between glucose in subsites +1/+2.

With *Aspergillus aculeatus* EGII (XG5, Cel12B), glucose is seen to be the main low molecular weight product (Tables 11 and 12). Lichenase treatment of samples with little degradation of beta-glucan by EG II gives increase of cellobiose, cellotriose and laminaribiose but not glucose. This indicates that beta-1,3 bonds are accepted between glucose units in subsites -5/-4, -4/-3 and +1/+2 but probably not -3/-2. Thus, the glucose liberated by EG II is probably released by exo-action on degradation products. The enzyme is able to hydrolyse both beta-1,4 and beta-1,3 bonds although beta-1,4 linkages seem to be preferred. After 20 hours with the highest enzyme concentration, the beta-glucan is seen to be almost totally degraded to glucose.

The *Aspergillus aculeatus* EG III (XG53, Cel12A) rapidly degrades the beta-glucan giving oligomers of DP4 (mainly Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu and Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu) and DP5 (mainly Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu but also some Glu(beta-1,4)Glu(beta-1,3)Glu(beta-1,4)Glu(beta-1,4)Glu and Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,4)Glu(beta-1,3)Glu) (Tables 13 and 14). After 20 hours with the highest enzyme concentration significant amounts of cellobiose, glucose and cellotriose are also formed. Lichenase treatment of samples gives increase of glucose, cellotriose and laminaribiose and especially cellobiose. This indicates that beta-1,3 bonds may be preferred between glucose units in subsites -4/-3 but are also accepted between -5/-4, -3/-2 and +1/+2. The enzyme is capable of degrading both beta-1,4 and beta-1,3 linkages.

Table 5: Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG I given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.10	0.28	0.35	3.68	15.45	40.96
Cel_2	0.40	0.93	1.51	13.80	27.32	28.76
Cel_3	0.69	1.64	2.38	9.91	6.00	0.00
Cel_4	0.25	0.48	0.68	2.37	0.85	0.00
Cel_5	0.00	0.40	0.35	1.66	0.11	0.00
Cel_6	0.00	0.00	0.00	0.00	0.00	0.00
Lam_2	0.00	0.00	0.13	0.07	0.06	2.12
DP_3	0.63	0.00	0.06	0.86	3.51	5.33
DP_4	0.00	0.00	0.08	1.29	4.40	4.13
DP_5	0.87	2.38	3.65	22.91	23.44	9.14
DP_6	1.12	2.66	4.50	16.08	4.16	3.41
DP>6	95.93	91.23	86.30	27.38	14.70	6.16

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 6. Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG I and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.14	0.17	0.45	5.15	16.24	43.66
Cel_2	3.38	4.99	10.09	36.73	43.18	35.48
Cel_3	1.24	3.31	5.26	14.14	6.42	0.17
Cel_4	0.21	0.79	1.39	3.79	0.90	0.00
Cel_5	0.00	0.16	0.62	1.18	0.84	0.00
Cel_6	0.00	0.00	0.00	0.00	0.00	0.00
Lam_2	2.95	2.43	3.58	9.90	7.53	4.99
DP_3	61.59	58.54	52.42	3.56	6.49	5.46
DP_4	20.92	19.72	16.97	5.19	6.01	4.58
DP_5	4.33	4.10	5.46	13.49	9.44	2.84
DP_6	2.23	2.88	2.75	4.84	1.00	2.47
DP>6	3.01	2.91	0.29	2.04	1.96	0.34

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 7: Results upon degradation of barley beta-glucan with endoglucanase *Humicola insolens* EG III given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.00	0.08	0.06	0.30	0.25	0.68
Cel2	0.00	0.00	0.00	0.08	0.01	0.53
Cel3	0.00	0.00	0.00	0.00	0.00	0.00
Cel4	0.00	0.00	0.00	0.00	0.03	0.00
Cel5	0.00	0.00	0.00	0.00	0.00	0.00
Cel6	0.00	0.00	0.00	0.00	0.00	0.00
Lam2	0.00	0.00	0.00	0.00	0.03	0.24
DP3	1.07	0.00	0.00	0.66	0.05	0.00
DP4	0.00	0.14	0.53	5.99	7.95	39.08
DP5	0.78	0.00	0.75	4.50	6.68	25.08
DP6	0.00	0.00	0.36	1.26	1.92	7.08
DP>6	98.15	99.78	98.29	87.22	83.09	27.31

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 8: Results upon degradation of barley beta-glucan with endoglucanase *Humicola insolens* EG III and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.15	0.29	1.37	6.70	14.43	13.80
Cel2	0.32	0.19	0.44	0.21	0.24	0.90
Cel3	1.03	0.00	2.01	0.93	0.45	0.15
Cel4	0.00	0.00	0.00	0.00	0.03	0.00
Cel5	0.80	0.00	0.00	0.00	0.00	0.00
Cel6	0.00	0.00	0.00	0.00	0.00	0.00
Lam2	4.10	1.26	2.10	7.43	13.20	13.77
"Lic3"	59.81	65.09	61.00	47.76	40.48	56.20
"Lic4"	22.63	23.12	21.54	18.78	7.08	9.72
"Lic5"	4.24	4.45	4.92	10.59	15.64	2.88
"Lic6"	3.91	2.82	3.67	3.28	2.90	2.05
"Lic7"	3.00	2.79	2.93	4.32	5.54	0.55

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 9: Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG IV given as weight % of degradation products. Glu: Glucose.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.00	0.00	0.00	0.13	0.63	0.66
Cel_2	0.14	0.38	1.07	7.02	5.51	12.11
Cel_3	0.09	0.19	0.77	2.89	1.72	1.02
Cel_4	0.81	0.20	0.34	1.10	0.55	0.13
Cel_5	0.15	0.28	0.30	0.00	0.00	0.00
Cel_6	0.00	0.29	0.00	0.00	0.00	0.00
Lam_2	0.00	0.00	0.00	0.00	0.04	0.16
DP_3	0.00	0.00	0.00	0.68	0.00	0.11
DP_4	0.00	0.00	0.00	1.03	1.83	12.77
DP_5	0.18	0.21	0.07	0.59	0.71	3.25
DP_6	0.00	0.13	0.26	5.78	6.04	2.44
DP>6	98.63	98.32	97.20	80.77	82.96	67.36

Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 10: Degradation products of barley beta-glucan with endoglucanase *Humicola insolens* EG IV and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	0.1	10	10	10
Incubation time (hours)	1	2.5	21.5	1	2.5	21.5
Glu	0.07	0.05	0.09	1.83	2.93	7.24
Cel_2	0.40	0.50	1.97	4.53	7.23	19.84
Cel_3	1.45	2.05	5.59	11.84	12.00	6.94
Cel_4	0.81	1.13	1.90	1.48	0.57	0.08
Cel_5	0.00	0.00	0.00	0.00	0.00	0.00
Cel_6	0.00	0.00	0.00	0.00	0.00	0.00
Lam_2	2.10	3.92	3.82	5.43	7.41	11.87
DP_3	63.45	61.92	60.26	54.03	47.65	30.59
DP_4	22.95	23.32	21.96	16.11	11.03	15.88
DP_5	4.97	4.99	3.46	3.03	2.51	4.01
DP_6	3.82	0.20	0.49	0.11	3.04	1.00
DP>6	0.00	1.93	0.47	1.60	5.62	2.55

Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 11: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG II (XG5, Cel12B) given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.16	0.16	16	16
Incubation time (hours)	1	20	1	20
Glu	0.17	2.30	33.64	99.25
Cel_2	0.00	0.00	0.54	0.00
Cel_3	0.00	0.00	0.60	0.00
Cel_4	0.00	0.00	0.52	0.00
Cel_5	0.00	0.00	0.11	0.00
Cel_6	0.00	0.00	0.00	0.00
Lam_2	0.00	0.12	2.97	0.00
DP_3	0.00	0.45	0.09	0.16
DP_4	0.00	0.06	1.85	0.02
DP_5	0.00	0.20	1.69	0.16
DP_6	0.00	0.28	4.58	0.00
DP>6	99.83	96.59	53.42	0.41

Glu: Glucose. Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 12: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG II (XG5, Cel12B) and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.016	0.016	1.6	1.6
Incubation time (hours)	1	20	1	20
Glu	0.20	2.21	26.22	99.53
Cel_2	4.88	0.61	1.31	0.00
Cel_3	3.76	3.44	4.10	0.00
Cel_4	0.00	0.20	0.82	0.00
Cel_5	0.00	0.88	0.00	0.00
Cel_6	0.00	0.00	0.00	0.00
Lam_2	0.17	2.17	9.95	0.00
DP_3	61.15	59.72	36.11	0.27
DP_4	23.43	21.49	14.35	0.04
DP_5	3.82	3.83	3.38	0.16
DP_6	0.08	2.52	2.20	0.00
DP>6	2.51	2.94	1.55	0.00

Glu: Glucose. Celi: Cellooligo of DP i. Lam2: Laminaribiose. DPi: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

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Table 13: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG III (XG53, Cel12A) given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	10	10
Incubation time (hours)	1	20	1	20
Glu	0.05	0.23	1.42	13.28
Cel_2	0.09	0.78	4.20	20.68
Cel_3	0.15	1.21	2.69	7.57
Cel_4	0.17	0.91	1.19	0.00
Cel_5	0.08	0.00	0.00	0.00
Cel_6	0.00	0.00	0.00	0.00
Lam_2	0.00	0.15	0.25	0.03
DP_3	0.33	0.16	0.00	0.42
DP_4	0.28	8.71	40.77	33.42
DP_5	1.24	15.49	30.18	20.94
DP_6	0.79	6.69	0.26	1.65
DP>6	96.83	65.67	19.04	2.01

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

Table 14: Degradation products of barley beta-glucan with endoglucanase *Aspergillus aculeatus* EG III (XG53, Cel12A) and subsequent lichenase degradation given as weight % of degradation products.

Enzyme dosage (microgram/ml)	0.1	0.1	10	10
Incubation time (hours)	1	20	1	20
Glu	1.08	6.84	7.12	16.22
Cel_2	3.37	16.31	21.82	30.46
Cel_3	3.90	5.25	4.40	7.66
Cel_4	0.70	2.35	0.65	0.03
Cel_5	0.58	0.00	0.00	0.10
Cel_6	0.00	1.22	0.00	0.00
Lam_2	4.12	16.93	16.24	5.07
DP_3	57.22	33.12	6.11	0.99
DP_4	18.69	12.16	38.91	35.81
DP_5	4.39	1.46	2.41	2.62
DP_6	3.44	2.16	0.26	0.78
DP>6	2.51	2.19	2.08	0.26

Glu: Glucose. Cel_i: Cellooligo of DP i. Lam_2: Laminaribiose. DP_i: Oligosaccharide of DP i with a single beta-1,3 bond and the rest beta-1,4 bonds between the glucose units. DP>6: Oligosaccharide consisting of more than 6 glucose units.

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Example 4. Mashing and filtration performance

A conventional standard treatment of Ultraflo® 2.7 mg EP/kg dry matter (dm) grist (index 1,000) was compared to an experimental treatment with Ultraflo® 1.4 mg EP/kg dm grist supplemented with various endoglucanases. A dosage of 0.2 g Ultraflo® /kg DM grist
10 equals 2.7 mg enzyme protein/kg dm grist.

Table 15. Effect of *Humicola insolens* EG I endoglucanase (Cel 7b, GH 7) and *Humicola insolens* EG V endoglucanase, (Cel 45a, GH45).

	Beta-glucan	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	-
Ultraflo® 1.4 mg EP/kg dm + HI EG I 1,25 mg EP/kg dm	1.184	0.997	1.032	0.904	-
Ultraflo® 1.4 mg EP/kg dm + HI EG V 1,25 mg EP/kg dm	2.986	0.996	1.033	0.865	-
Ultraflo® 1.4 mg EP/kg dm + HI EG I 8 mg EP/kg dm	**0.377**	0.992	1.021	0.962	** beta - glucan
Ultraflo® 1.4 mg EP/kg dm + HI EG V 8 mg EP/kg dm	3.262	1.000	1.055	0.865	-

Beta-glucan (n=4), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

Ultraflo® 1.4 mg EP/kg dm supplemented with *H. insolens* EG I, Cel 7b (GH 7) 8 mg EP/kg dm reduced beta-glucan compared to the standard treatment (index 1.000).

Table 16. Effect of *Humicola insolens* EGIII endoglucanase, (Cel 12a, GH12) and *Humicola insolens* EG IV endoglucanase, (Cel 5a, GH12).

	Beta-glucan	OD	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1.000	-
Ultraflo® 1.4 mg EP/kg dm + HI EG IV 1,25 mg EP/kg dm	3.019	0.975	1.002	1.002	0.979	-
Ultraflo® 1.4 mg EP/kg dm + HI EG III 1,25 mg EP/kg dm	0.628	0.949	1.000	0.999	0.957	-
Ultraflo® 1.4 mg EP/kg dm + HI EG IV 8,0 mg EP/kg dm	2.045	1.013	0.999	1.006	1.085	-
Ultraflo® 1.4 mg EP/kg dm + HI EG III 8,0 mg EP/kg dm	**0.341**	**0.937**	1.003	**0.938**	**1.085**	*** beta-glucan, viscosity, filterability

Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

The *H. insolens*, endoglucanase III, (Cel 12a, GH12) and Ultraflo® 1.4 mg EP/kg dm reduced the beta-glucan, O.D and viscosity while also improving filterability compared to the standard treatment.

Table 17. Effect of *Thermoascus aurantiacus* endoglucanase (GH 5).

	Beta-glucan	OD	Extract	Viscosity	Filterability	Best Perfor-ming
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1.000	
Ultraflo® 1.4 mg EP/kg dm + AT EG 5 1,25 mg EP/kg dm	1.627	1.065	1.002	1.015	1.037	
Ultraflo® 1.4 mg EP/kg dm + AT EG 8 mg EP/kg dm	**0.432**	1.033	1.001	1.017	1.000	** beta - glucan

Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

Ultraflo® 1.4 mg EP/kg dm supplemented with the *T. aurantiacus* endoglucanase BG025 (GH 5) reduced the beta-glucan level significantly compared to the standard treatment.

A conventional standard treatment of Ultraflo® 0.2 g/kg DM grist (index 1,000) was compared to an experimental treatment with Ultraflo® 0.1 g/kg DM grist supplemented with various xylanases.

None of the two GH 11, type B xylanases from the fungi *Bh* and *Cc* had any positive effect on beta-glucan, OD, Extract recovery, viscosity or filterability.

Table 18. Effect of *Bh* xylanase B (GH 11, type B) & *Cc* xylanase II (GH 11 type B).

	Beta-glucan	OD	Extract	Viscosity	Filterability	Best Perfor-ming
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1,000	-
Ultraflo® 1.4 mg EP/kg dm + BH XYL 0,7 mg EP/kg dm	3.223	1.100	1.000	1.032	1,082	-
Ultraflo® 1.4 mg EP/kg dm + CC XYL II 0,7 mg EP/kg dm	3.279	1.025	0.998	1.028	1,012	-
Ultraflo® 1.4 mg EP/kg dm + BH XYL B 5 mg EP/kg dm	3.231	1.025	1.000	1.048	1,035	-
Ultraflo® 1.4 mg EP/kg dm + CC XYL II 5 mg EP/kg dm	3.213	1.038	1.003	1.030	1,082	-

Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

Table 19. Effect of *Aspergillus aculeatus* xylanase I (GH10, type A) and *Myceliophotora thermophila* xylanase III (GH 11, type B).

	Beta-glucan	OD	Extract	Viscosity	Filterability	Best Per-for-ming
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	1,000	-
Ultraflo® 1.4 mg EP/kg dm+ AA XYL I 5 mg EP/kg dm	3.401	1.125	1.006	**0.981**	**1,143**	*** Visco sity, filtera bility
Ultraflo® 1.4 mg EP/kg dm+ MT XYL II 5 mg EP/kg dm	3.740	0.990	1.005	1.019	0,939	-
Ultraflo® 1.4 mg EP/kg dm+ AA XYL I 0,7 mg EP/kg dm	3.894	1.010	1.006	1.006	0,980	-
Ultraflo® 1.4 mg EP/kg dm+ MT XYL II 0,7 mg EP/kg dm	3.218	0.927	1.007	1.020	0,776	-

Beta-glucan (n=4), OD (n=2), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

Table 20. Effect of *Thermomyces lanuginosus* xylanase (GH 11, type B) and *Aspergillus aculeatus* xylanase II (GH10, type A).

	Beta-glucan	Extract	Viscosity	Filterability	Best Performing
Ultraflo® 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	-
Ultraflo® 1.4 mg EP/kg dm + AA XYL II 0,7 mg EP/kg dm	2.296	1.002	0.985	0.981	-
Ultraflo® 1.4 mg EP/kg dm + TL XYL 0,7 mg EP/kg dm	2.375	0.994	1.015	0.868	-
Ultraflo® 1.4 mg EP/kg dm + AA XYL II 5 mg EP/kg dm	2.152	1.000	0.970	1.075	*** Viscosity, filterability
Ultraflo® 1.4 mg EP/kg dm + TL XYL 5 mg EP/kg dm	2.357	1.004	1.032	0.906	-

Beta-glucan (n=4), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

The *Aspergillus aculeatus* xylanase I and *Aspergillus aculeatus* xylanase II reduced viscosity as well as improved filterability compared to the standard treatment.

A conventional standard treatment of Ultraflo® 0.2 g/kg dm grist (index 1.000) was compared to an experimental treatment with Viscozyme 0.1 g or 0 g/kg dm grist supplemented with *Aspergillus aculeatus* xylanase II and various endoglucanases.

Table 21. Effect of *Aspergillus aculeatus* betaglucanase EGI (XG 5) and *Aspergillus aculeatus* endoglucanase EGIII (XG53) in combination with *Aspergillus aculeatus* xylanase II and/or the Viscozyme® endoglucanase composition.

	Beta-glucan	Extract	Viscosity	Filterability	Best Performing
Ultraflo®: 2.7 mg EP/kg dm	1.000	1.000	1.000	1.000	
AA EG II 4 mg EP/kg dm AA XYL II 4 mg EP/kg dm Viscozyme® 1.7 mg EP/kg dm	5.795	0.997	0.981	1.140	
AA EG III 1 mg EP/kg dm AA XYL II 4 mg EP/kg dm Viscozyme® 1.7 mg EP/kg dm	2.631	1.000	**0.964**	**1.118**	** viscosity, filterability
AA EG III 2 mg EP/kg dm AA XYL II 4 mg EP/kg dm Viscozyme® 1.7 mg EP/kg dm	1.317	1.003	0.955	1.011	
AA EG III 4 mg EP/kg dm, AA XYL II 4 mg EP/kg dm	**0.918**	1.004	**0.956**	**1.236**	*** beta -glucan, viscosity, filterability
AA EG II 8 mg EP/kg dm AA XYL II 4 mg EP/kg dm	6.601	1.003	0.977	1.096	

Beta-glucan (n=4), Extract % (n=4, based on dry matter), Viscosity (n=4, conv. 8,6° Plato, cP), Filterability (n=2) after 10 min

A combination of *Aspergillus aculeatus* Xylanase II and *Aspergillus aculeatus* endoglucanase EG III had a significant effect on beta-glucan, viscosity and filterability.

Table 22. Comparison of increasing amounts of Viscozyme® and of a composition of the present invention. Absolute values.

	Beta-glucan	OD	Extract %	Viscosity
Viscozyme® 3.6 mg EP/kg dm	189	0.030	85.0	1.38
Viscozyme® 9 mg EP/kg dm	155	0.030	85.0	1.35
Viscozyme® 13.5 mg EP/kg dm	127	0.031	85.2	1.34
Viscozyme® 18 mg EP/kg dm	101	0.028	85.5	1.32
Viscozyme® 27 mg EP/kg dm	75	0.030	85.7	1.30
Viscozyme® 3.6 mg EP/kg dm AA EG III 2 mg EP/kg dm AA XYL II 4 mg EP/kg dm	0	0.030	85.8	1.22

Beta-glucan (mg/l, n=4), OD (n=2), Extract % (n=4, extract in dry malt, % (m/m)), Viscosity (n=4, conv. 8,6° Plato, cP),

A composition comprising Viscozyme® 3.6 mg EP/kg dm, *Aspergillus aculeatus* EG III 2 mg EP/kg dm, and *Aspergillus aculeatus* Xylanase II 4 mg EP/kg dm had a significantly more positive effect on beta-glucan, OD, extract recovery, and viscosity than had a dosage of 7.5 times the conventional standard dosage of Viscozyme® (Std. dosage = 3.6 mg EP/kg dm) (Table 22).

Example 5. Quantification of protein bands in SDS-PAGE gels.

The enzyme composition was diluted 250 times in deionized water and loaded onto

a 4-20% Tris-glycine SDS-PAGE gel (Nu Page, Invitrogen) and the electrophoresis was conducted as described by the manufacturer.

After electrophoresis the gel was stained with GelCode Blue (Pierce) o/n and subsequently decolorized in water to the background became clear.

The resulting gel was then scanned using a densitometer and analyzed by the ImageMaster™ v. 1 0 software from Amersham Biosciences following the protocol from the manufacturer. The results are expressed as %band density of total density in a given lane.

The total amount of protein in the enzyme samples were measured using the Micro BCA kit from Pierce using the protocol supplied with the kit.